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Brain mediators of cardiovascular responses to social threat, Part I: Reciprocal dorsal and ventral sub-regions of the medial prefrontal cortex and heart-rate reactivity

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Abstract

Social threat is a key component of mental "stress" and a potent generator of negative emotions and physiological responses in the body. How the human brain processes social context and drives peripheral physiology, however, is relatively poorly understood. Human neuroimaging and animal studies implicate the dorsal medial prefrontal cortex (MPFC), though this heterogeneous region is likely to contain multiple sub-regions with diverse relationships with physiological reactivity and regulation. We used fMRI combined with a novel multi-level path analysis approach to identify brain mediators of the effects of a public speech preparation task (social evaluative threat, SET) on heart rate (HR). This model provides tests of functional pathways linking experimentally manipulated threat, regional fMRI activity, and physiological output, both across time (within person) and across individuals (between persons). It thus integrates time series connectivity and individual difference analyses in the same path model. The results provide evidence for two dissociable, inversely coupled sub-regions of MPFC that independently mediated HR responses. SET caused activity increases in a more dorsal pregenual cingulate region, whose activity was coupled with HR increases. Conversely, SET caused activity *decreases* in a right ventromedial/medial orbital region, which were coupled with HR increases. Individual differences in coupling strength in each pathway independently predicted individual differences in HR reactivity. These results underscore both the importance and heterogeneity of MPFC in generating physiological responses to threat.

Introduction

One of the most remarkable features of the mammalian nervous system is its ability to mount coordinated behavioral and physiological responses to environmental demands. For

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example, when environmental cues signal a potential threat to an organism's well being, the brain produces a coordinated set of behavioral, autonomic, and metabolic changes that promote an adaptive response. As Walter Cannon (Cannon, 1932) and many others since have described, output from the brain to the peripheral autonomic nervous system and endocrine system prepares us to respond rapidly and effectively to impending threats. For example, the classic "fight or flight" response involves increases in heart rate, blood flow to the limbs, pupil dilation, slowed digestion, and other changes (Bandler, Keay, Floyd, & Price, 2000; Obrist, 1981). The brain systems that regulate the various organ systems of the body have evolved from survival-related brainstem circuits, but also include cortical and subcortical systems central to social and emotional processes (Bandler & Shipley, 1994; Craig, 2003; Porges, 2003). Thus, understanding these brain-body information transfer systems may provide clues into the neural organization of social and emotional behavior and its consequences for the body.

Threat is one of the oldest and presumably most basic brain processes that strongly influences the body. Threat responses can be triggered by the presence of individual, simple cues (e.g., a light or tone) acting through defined circuitry in the amygdala, periaqueductal gray (PAG), and other regions (Davis, 1992; LeDoux, 2000). However, threat responses are much more often triggered by patterns of cues and conceptual knowledge stored in memory that fit together into a situational "schema," which strongly suggests the involvement of a more complex set of cortical and subcortical processes. For example, darkness, shadows that look like human forms, the sound of a mechanical click in the silence, and the knowledge that one is walking alone in a dangerous part of the city may all combine to trigger a schema that one might call "impending threat." Threat responses can also be triggered by social situations that involve complex appraisals of social relationships, including an individual's status, competence, and value in the eyes of others. Indeed, threats in modern human life are usually abstract and often related to the maintenance of our self-esteem, social status, and long-term prospects for mating and longevity. Threats generated by social or other cognitive processes are particularly under-studied in neuroscience, but they can offer important clues about the brain pathways involved in common types of threat in contemporary society.

The study of threat systems in the brain has important implications for health. While advantageous in the short term, threat responses that persist over time can have deleterious effects on the brain and body. Chronic perception of threat has been shown to increase the risk of heart disease (Bosma, 1998; Jain, Joska, Lee, Burg, & Lampert, 2001; Rozanski et al., 1988; Sheps, 2002), cause hippocampal deterioration (Smith, Makino, Kvetnansky, & Post, 1995; Stein-Behrens, Mattson, Chang, Yeh, & Sapolsky, 1994; Watanabe, Gould, & McEwen, 1992) and impairments in declarative memory (McEwen & Sapolsky, 1995), promote pro-inflammatory immune responses (Kiecolt-Glaser & Glaser, 2002), and contribute to cognitive and physical aging (Mcewen, 2007), among other adverse effects. Both threat states and their negative connotations for health are captured in early concepts of "stress" (Selye, 1956) and the more recent concept of "allostatic load" (Mcewen, 2007)—the notion that a) the brain actively maintains homeostasis through the activation of brain, autonomic, and endocrine systems, and b) chronic load on these systems by persistent threat has deleterious effects on the brain and body, contributing to a variety of health problems (Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002).

The brain mechanisms underlying social threat responses are just now beginning to be addressed using neuroimaging techniques. Much progress has been made in understanding the neural substrates of threat and stress in animals, but surprisingly little is known about how social and performance "stressors" affect the human brain. The goal of the present study, and its companion (Wager et al., submitted), was to investigate the cortical and subcortical systems involved in generating physiological responses to a well-validated

laboratory manipulation of social threat. These studies complement and extend a small but growing literature on the neural bases of social and performance stress (Critchley, 2003; Dedovic et al., 2005; Eisenberger, Taylor, Gable, Hilmert, & Lieberman, 2007; P. J. Gianaros, F. M. Van Der Veen, & J. R. Jennings, 2004; Kern et al., 2008).

Speech preparation as a model of social threat

In human laboratory studies social status-related threats have been studied in the context of *social evaluative threat* (SET)—the condition of being judged unfavorably by other individuals in a public setting. SET has been shown to be the most potent human laboratory elicitor of a canonical feature of stress in animal models: the hypothalamic-pituitary-adrenal (HPA) axis response (Dickerson & Kemeny, 2004; Kirschbaum, Pirke, & Hellhammer, 1993). Threats to the 'social self' in particular elicit HPA-axis responses (Dickerson, Gruenewald, & Kemeny, 2004). Remarkably, inter-correlated autonomic, endocrine, and immune changes are produced by even acute SET challenges (Cacioppo, 1994; Cohen et al., 2000; Kiecolt-Glaser, Cacioppo, Malarkey, & Glaser, 1992; Sgoutas-Emch et al., 1994). These effects are clinically relevant as well. SET challenges in patients with coronary artery disease have been shown to induce myocardial ischemia (Rozanski et al., 1988) and affect clinical measures of cardiac dysfunction, including left ventricular ejection fraction (LVEF) (Jain et al., 2001; Jain et al., 1998). Ischemic responses to SET have been shown to predict the incidence of fatal and non-fatal cardiac events over a 5-year follow-up (Jiang et al., 1996; Sheps, 2002).

In this study, we assess fMRI activity elicited by public speech preparation, a component of well-studied laboratory SET challenges, and its relationship with heart rate (HR). Both preparing and giving a speech before a critical audience induces robust cardiovascular engagement, including increased blood pressure and heart rate (HR) (Berntson et al., 1994; Cacioppo et al., 1995; Gramer & Saria, 2007; Tugade & Fredrickson, 2004; Uchino, Cacioppo, Malarkey, & Glaser, 1995) that results from both increased sympathetic output and reduced parasympathetic output to the heart (Berntson et al., 1994). Public speaking stressors have produced larger cardiac chronotropic responses than math performance and reaction-time based stressors (Al'Absi et al., 1997; al'Absi, Bongard, & Lovallo, 2000; Berntson et al., 1994), though HR responses are comparable whether participants are giving the speech or only preparing it (Feldman, Cohen, Hamrick, & Lepore, 2004; Gramer & Saria, 2007; Waugh, Panage, Mendes, & Gotlib, 2008).

We focused on HR as an outcome measure for several reasons. First, HR increases are robustly elicited by SET, though they vary across individuals (Berntson et al., 1994). They are substantially more robust than more pure measures of sympathetic and parasympathetic activity collected over short time intervals (Berntson et al., 1994; Cacioppo et al., 1994). Studies of stressor-induced HR reactivity have estimated its internal consistency above alpha = .95 and test-retest reliability around r = .6 after one year (Cacioppo, 1994; Uchino et al., 1995). Second, they can be measured on a roughly second-by-second basis, providing the ability to analyze effective connectivity among key brain regions and HR across time. Third, HR reactivity and cardiovascular reactivity more generally predict other health-related effects of stressors on the body. Cardiovascular reactivity is heritable (Carroll, Hewitt, Last, Turner, & Sims, 1985) and is correlated with stressor-induced changes in cortisol release (Al'Absi et al., 1997; Lovallo, Pincomb, Brackett, & Wilson, 1990) and immune function (Cacioppo, 1994; Cacioppo et al., 1995; Sgoutas-Emch et al., 1994; Uchino et al., 1995). Finally, cardiovascular reactivity measures related to HR, such as heart-rate variability and LVEF, are risk factors for cardiac dysfunction and mortality (Thayer & Lane, 2007).

Cortical and subcortical systems linked to threat responses

The most likely locations for brain generators of cardiovascular and other peripheral responses to SET are in the medial prefrontal cortex (MPFC), which projects reciprocally to a set of interconnected "limbic" cortical regions and subcortical nuclei, including the insula, medial temporal lobes, amgydala, ventral striatum (ventral caudate and putamen), mediodorsal thalamus, hypothalamus, and PAG, as well as other important brainstem nuclei (An, Bandler, Ongür, & Price, 1998; Bandler et al., 2000; Bandler & Shipley, 1994; Barbas, Saha, Rempel-Clower, & Ghashghaei, 2003; Hsu & Price, 2007; Kondo, Saleem, & Price, 2003, 2005; Price, 1999; Saleem, Kondo, & Price, 2008). MPFC has been broadly associated with emotional processes (T. Wager et al., 2008), with dorsomedial and pregenual regions linked to PAG activation, and tasks that engage self-evaluation (Northoff et al., 2006).

In human imaging studies, the dorsal cingulate/MPFC has been linked consistently with stress-induced increases in HR and blood pressure (Critchley, Corfield, Chandler, Mathias, & Dolan, 2000; Critchley et al., 2003; Critchley, Tang, Glaser, Butterworth, & Dolan, 2005; P. Gianaros, F. M. Van Der Veen, & J. R. Jennings, 2004; Gianaros, Jennings, Sheu, Derbyshire, & Matthews, 2007; Gianaros et al., 2008a) and cortisol (Eisenberger et al., 2007). More rostral and ventral areas have been associated with reduced cortisol reactivity (Eisenberger et al., 2007; Kern et al., 2008), implying a role in successful regulation or protection from stress reactivity. These studies mark an important milestone in the interrelation of human brain activity and physiology, and have confirmed and extended findings from animal models implicating the ventromedial prefrontal cortex (vmPFC), lateral orbitofrontal cortex (OFC), anterior cingulate (ACC), and anterior insula (aINS)—the same regions thought to be most critical for emotional appraisal—in physiological responses to social threat.

One limitation is that nearly all of the studies cited above (and most others) have relied primarily on between-subject correlations to make inferences about brain-physiology relationships. For example, Eisenberger et al. (2007) related individual differences in cortisol responses to brain activity responses in a separate social exclusion task. Though a promising way to examine individual differences, such correlations do not take full advantage of the capability of fMRI to make many repeated measurements of brain activity over time (typically 200–1500 per individual). Thus, these studies are limited in power by the sample size (though the Eisenberger study was particularly large compared to other fMRI studies). In addition, between-subject correlations are subject to a number of confounds related to individual differences in age, neurovascular coupling, brain morphometry, and other variables. Other studies have assessed relationships between brain activity and physiological changes across time, and tested whether these relationships are consistent across participants (Critchley et al., 2005; P. J. Gianaros et al., 2004; Lane et al., 2009). For example, in a particularly large study, Gianaros et al. (2004) mapped brain regions in which task-evoked heart period changes across a series of working memory tasks correlated with variation in task-evoked brain activity.

In this study, we extend these results by using a new kind of analysis—multi-level mediation effect parametric mapping—that is specifically designed to link experimental manipulations, brain activity, and physiological output in a single path model. A single-level version of the model was used in (Wager, Hughes, Davidson, Lindquist, & Ochsner, 2008). One advantage of the multi-level model is that it can incorporate both within-subjects longitudinal effects across time and between-subjects effects of individual differences in the same model. The first, within-subject level of the two-level model utilizes the rapid sampling capabilities of fMRI to estimate brain-physiology relationships across time. The second, between-subject level captures how activity and connectivity relate to other measures of individual

differences. In addition, it can provide tests of mediation that standard general linear model-based analyses cannot.

We experimentally manipulated SET by asking participants to silently prepare a speech under time pressure (Figure 1A). Participants believed that they would have to give their speech (they did not), which would be audiotaped during scanning and judged later by fellow students. We monitored HR continuously during fMRI imaging, and our analyses focused on establishing pathways that link the experimental SET manipulation with variations in brain activity and HR.

The overall inference that a region is critical for generating HR responses to SET includes tests at two levels of analysis. The *first level* of analysis tests associations between SET, brain activity, and HR across time within individuals. At this level, a region involved in generating HR responses to threat should show the following three characteristics. Activity in a brain region should: 1) increase (or decrease) in response to the SET challenge (Path *a* in Figure 2); 2) predict HR changes over time, controlling for the SET manipulation (Path b in Figure 2); and 3) Mediate the SET-HR covariance. This latter criterion can be evaluated using a mediation test, which formally tests whether the brain region explains a significant proportion of the SET-HR covariance. The *second level* of analysis concerns HR reactivity. If a particular brain region is a mediator of the SET-HR relationship, and this relationship underlies individual differences in HR reactivity, then the first-level *a* and *b* path strengths should be predicted by HR reactivity. That is, for those who show robust HR increases to the SET challenge, brain activity in mediating regions should be more strongly associated with both SET and HR. Inferences about brain regions that link social threat with autonomic activation draw on each of these five hypotheses (three related to dynamic co-variation across time and two related to individual differences.)

Methods

Participants

Thirty healthy, right-handed, native English speakers were recruited at the University of Michigan (mean age 20.3 years, 10 males) and participated in this experiment. Potential participants were initially pre-screened for scoring in the upper or lower quartile of an emotional resilience measure (ER-89)(Block & Kremen, 1996). However, none of the results presented in this paper were related to this personality trait ($p > .5$), so the two subgroups were combined in all analyses. Resilience-related results from this sample on a different task are presented elsewhere (Waugh, Wager, Fredrickson, Noll, & Taylor, 2008). Participants were excluded who reported a prior history of neurological or psychiatric illness, current or prior psychoactive medication, claustrophobia, or other standard contraindications for fMRI, and were asked to abstain from tobacco and caffeine use 24 hours prior to scanning. All participants gave written informed consent as approved by the University of Michigan institutional review board. Two participants were excluded due to excessive head motion (> 3 mm), two were excluded because sufficient anatomical warping quality could not be achieved, and two additional participants did not have complete HR data, leaving a final sample of 24 participants.

Procedure and fMRI task design

A schematic description of the 7-min long task is depicted in figure 1A. After an initial anatomical scan, participants were instructed that they were to prepare a speech that would be audiotaped in the scanner and then judged by fellow students on persuasiveness, organization and intellectual quality. Participants were given headphones with a microphone attached. They were also told that there was a slight possibility that they would not have to

give the speech. After reading these instructions, the participants were told to relax and focus on a fixation cross for two minutes, during which we acquired baseline physiological and fMRI data. At the end of two minutes, the speech topic “Why am I a good friend” was presented for 15 seconds, and the participants were told they would have two minutes to prepare their speech, which should be 7 min long, and they should prepare to speak for the full 7 min. After a 2-min preparation period, participants were instructed that they were randomly selected to not give a speech, and asked to relax for the remaining 2.5 minutes of fMRI scanning. We presented computerized instructions during all phases using E-prime software (Psychology Software Tools).

Although this design has been used several times in psychophysiology studies, this is an unusual design for an fMRI study because it involves only a single, relatively sustained SET challenge. Allowing participants a sustained 2-min period to develop emotional and physiological responses was a key design feature. Because most people cannot easily switch rapidly among emotional states, traditional block or event-related designs dilute emotional experience in favor of repeated occurrences of artificially manipulated epochs. The present task employed a novel analysis method, mutli-level mediation especially suited for capturing brain activity reflecting a more ecologically-valid emotional experience. We discuss this type of design more fully in the companion paper (Wager et al., submitted).

Data acquisition and preprocessing

Heart rate was collected continuously during scanning using a photo-plethysmograph on the left index finger (100 Hz sampling). Using customized software from the James Long Company, we first identified and removed outliers from the data (using a custom algorithm blind to task condition). Inter-beat intervals were then calculated from the remaining R-waves, and HR was averaged into 2 s bins (the scan repetition time, TR). HR reactivity was calculated as each participant’s mean HR during speech preparation (from the presentation of the speech topic to the presentation of the “relax” cue), compared with pre- and post-stress rest periods.

MRI images were collected on a 3.0T GE whole-body scanner (GE Medical Systems). Structural images were acquired using high-resolution T1 spoiled gradient recall images (SPGR) for anatomical localization and warping to a standard space. Functional blood-oxygen-level-dependent (BOLD) images were acquired with a T2*-sensitive spiral in-out sequence (Glover & Law, 2001) (TR = 2000 ms, TE = 40 ms, flip angle = 90°, 24 slices in ascending sequential sequence, 4.5 × 3.4375 × 3.475 mm voxels). An LCD projector displayed stimuli on a back-projection screen placed in the scanner room.

Functional images were subjected to a standard preprocessing sequence. Slice-timing acquisition correction using sync interpolation was performed using custom software written by Dr. Luis Hernandez, and realignment of the functional images to correct for head movement was performed using the Automated Image Registration tools (Woods, Grafton, Holmes, Cherry, & Mazziotta, 1998). The remaining preprocessing steps were performed using the Statistical Parametric Mapping analysis package (SPM2, Wellcome Department of Cognitive Neurology, London, UK). SPGR images for each participant were coregistered to the mean functional image. SPGR images were normalized to the anatomical space of the 152-brain template provided by the Montreal Neurological institute (MNI), and parameters were applied to the functional images. Finally, the normalized functional images were smoothed with an 8-mm full-width at half-maximum Gaussian smoothing kernel. This set of data is referred to as “raw” data in analyses and figures.

Prior to analysis, multiple regression was used to estimate and remove linear effects of a number of known nuisance covariates (see Figure 1B). These included, for each participant,

a) 6 estimated head movement parameters from realignment, their mean-zeroed squares, their derivatives, and squared derivatives; b) the whole-brain global signal time series (Vincent et al., 2006) (note that this is not the same as "global scaling" in SPM software and does not suffer from the same problems because it is not a re-scaling of the data (Aguirre, Zarahn, & D'Esposito, 1998)); c) indicator vectors for outlier time points identified based on their multivariate distance from the other images in the sample; and d) linear drift across time. These covariates were removed prior to analysis to ensure that the main analyses were not confounded by these variables. Global outliers (c above) were identified by computing both the mean and the standard deviation of values in each image for each slice. Mahalanobis distances for the matrix of mean values (one per slice) × functional volumes were computed, and images with a value above 3 standard deviations were considered outliers. The same procedure was used for standard deviation values. Typical numbers of outliers ranged between zero and four images per participant. All analyses described below were conducted on data after removing these covariates. Analyses conducted separately on the "raw" preprocessed data showed similar, but less spatially specific, effects (data not shown).

Statistical analysis: Multi-level path modeling

The multi-level path modeling approach we employed assesses relationships between experimental manipulations (*X*), brain activity (*M*), and physiology (*Y*) in the context of a single structural equation model (see Figure 2, and Supplementary Methods 1 for details). Unlike most standard structural models, though, it was formulated to test within-subjects relationships between *X*, *M*, and *Y* in a two-level mixed-effects framework. Because it is a mixed-effects model, it incorporates both "first-level" (within-subjects) and "second-level" (between-subjects) effects (individual differences in brain responses and HR reactivity). The advantages of using the structural model over a standard GLM approach are that: 1) It can provide tests of mediation effects; 2) It estimates several GLM equations in the context of a single path model, making it easy to localize regions that show a pattern of interest across multiple effects; and 3) within-subject measurement error is taken into account when conducting group analyses, providing increased efficiency if data quality is better for some subjects.

In this experiment, the initial variable (*X*) in the path model is experimentally manipulated SET (i.e., the contrast vector [SpeechPrep – (Pre + Post Baselines)], with a value of 0 during baseline periods and 1 during speech preparation). Pre- and post-threat baselines were not reliably different physiologically. The mediating variable (*M*) is the time series of brain activity in a single voxel, for each subject. The outcome variable (*Y*) is HR across time, for each subject. In addition, participants' HR reactivity scores were entered as a second-level predictor.

The standard analysis of experimental effects on fMRI activity (i.e., the [SpeechPrep – Baseline] contrast) is captured by the X->M relationship in the model, which we refer to as Path *a* (see Figure 2). A second effect of interest is the association between brain activity (*M*) and HR (*Y*), which we refer to as Path *b*. As is standard in directed path models, Path *b* is assessed while controlling for *X* (SET), so that any relationships between brain activity and HR cannot be attributed to the experimental manipulation as a third variable (Baron & Kenny, 1986;MacCorquodale & Meehl, 1948)—i.e., the brain-heart connectivity is not a side-effect of the common influence of SET. This analysis would typically be undertaken in a separate "effective connectivity" analysis. Finally, a *mediation test* provides inference on whether the inclusion of brain activity (*M*) in the model accounts for a substantial amount of the SET (*X*) to HR (*Y*) relationship. This is equivalent to testing the product of the path coefficients *a***b*.

Inferences at the second (group) level provide a "random effects" test of significance for each of these within-subjects effects. In addition, the model provides inferences about whether the strength of each effect (*a*, *b*, and *a*b*) is predicted by individual differences in HR reactivity. These effects would typically be estimated in separate brain-behavior correlation analyses.

Analyses are still performed using data from each brain voxel in a separate analysis (i.e., voxels are treated independently), so that the full path model and effects of interest are tested substituting each brain voxel's data for *M1*. This approach, which we refer to as Mediation Effect Parametric Mapping (T. D. Wager et al., 2008), retains the flexibility of the statistical parametric mapping approach in locating voxels that show particular effects of interest, but extends it to evaluating effects of interest in a simple structural equation model. In order to correct for multiple comparisons, we calculated the experiment-wise false discovery rate (FDR) (Genovese, Lazar, & Nichols, 2002) at $q < .05$ across all effects of interest, including the first- and second-level *a*, *b*, and *a*b* coefficients (equivalent to P < . 00037 uncorrected).

Analysis strategy—In this paper, we used a whole-brain, voxel-wise search to identify regions that showed overlapping Path *a* (SET responses) and Path *b* (HR connectivity) effects at the first level (Figure 2). We also report results from voxel-wise search for second-level, HR reactivity effects. Thus, inferences localizing regions related to both SET and HR ([Path *a*, Path *b*] overlap regions, Figure 3) were drawn from a multi-voxel brain mapping approach, with correction for multiple comparisons.

We conducted additional analyses on first-level [Path *a*, Path *b*] overlap regions and amygdala regions of interest (ROIs) defined *a priori* to characterize the nature and pattern of effects in these regions (Figure 3). We identified four sub-regions of the amygdala defined by Amunts et al. (Amunts et al., 2005) and labeled in the SPM anatomy toolbox v1.5 (Eickhoff et al., 2005), including the left and right corticomedial group (superior amgydala) reported most frequently in imaging studies of emotion (T. Wager et al., 2008) and the larger left and right basolateral complex.

Within these regions, we conducted two kinds of analyses. For these analyses, fMRI data were averaged over contiguous voxels in each region within each participant, yielding a single brain time-series per region per participant. First, we tested the full multi-level path model on each region. For the [Path *a*, Path *b*] overlap regions, this analysis simply duplicated the Path *a* and Path *b* effects used to select voxels (thus, the results are merely descriptive), but the model provided additional new information on the mediation (*a*b*) effect (see below) and on the second-level relationships with HR reactivity. We tested separate path models that included only one brain region as a mediator (M) variable. We also tested a single path model that included multiple key [Path *a*, Path *b*] overlap regions in the same model. The results from both types of model were qualitatively identical, in that the significance of the path coefficients was not affected by whether other mediators were included in the model. Thus, we focus on the results of the combined path model, which demonstrates independent mediation by multiple brain regions.

[1] *In the present results, for simplicity, we identify regions that show significant a and b paths, indicating a relationship with speech preparation and an independent relationship with HR, and then conduct mediation tests on the resulting regions. However, qualitatively identical results were found when voxel-by-voxel maps were made of the overlap of a, b, and a*b effects.*

Results

Physiological effects of SET

HR changes over time were a primary outcome measure of the SET challenge. Compared with pre-threat baseline and post-threat recovery, speech preparation induced reliable increases in HR (9.54 beats per minute, BPM, $t = 4.88$, $p < .0001$), as shown in Figure 1C. This effect was significant when preparation was compared separately with each of the first (8.16 BPM, $t = 4.18$, $p = .0004$) and second baseline periods (10.92 BPM, $t = 5.27$, $p < .0001$). HR was lower during the post-preparation recovery period than during the first baseline (2.76 BPM, $t = 2.91$, $p = .008$)2. There were 22 degrees of freedom for each planned test. HR reactivity was uncorrelated with ego resilience ($r = .01$, $P > .5$) or other measures collected in the sample reported in Waugh et al. (2008), including sex, ethnicity, income, age, and several other trait personality measures (all $P > .09$).

Brain responses to SET (Path *a*)

Figure 2 shows a path diagram that links Speech Prep (the experimental manipulation of SET), brain activity, and HR variation. The first within-subjects effect we tested localized brain regions activated by the SET challenge ([SpeechPrep – Baseline], Path *a* in Figure 2). This contrast was expected to show significant responses in a broad set of regions, including those critical for generating autonomic responses, visual responses to task instructions, and others.

Increases in activity to Speech Prep were found most prominently in the MPFC, and in particular the pre-genual anterior cingulate cortex (pgACC), extending into the right caudate, as well as several temporal and occipital cortical regions (yellow/red in Figure 2A). All significant regions are reported in Table 1. Decreases in activity related to SET were found prominently in the OFC, as well as the right putamen, ventral anterior insula, temporal cortex, and posterior cingulate cortex.

The Path *a* effect at the second level tested the correlation between the [SpeechPrep – Baseline] effect and HR reactivity. The results are shown in Figure 2C and Table 1. Positive effects, indicating positive correlations with HR reactivity, were found in a subset of regions showing first-level effects, including pgACC, extending into the right caudate nucleus, and right ventral temporal cortex (shown in warm colors). Negative correlations between SET and HR reactivity were found in bilateral OFC and right putamen (shown in cool colors).

Brain correlates of HR (Path *b*)

The second test for identifying brain regions that mediate the response of threat on HR is to identify regions that predict HR changes, both across time (first-level) and at the individual differences level (second-level). The brain-HR relationship was tested controlling for the Speech Prep indicator—a standard technique in path modeling to establish a mediator-outcome (here, brain-HR) relationship independent of task demand— and is depicted as Path *b* in Figure 2. At the first level, brain time series were correlated positively with HR variations across time most prominently in the pgACC, extending into the rostral dorsal anterior cingulate (rdACC), vmPFC, and right caudate head, as well as in the left DLPFC (middle frontal gyrus) and right caudate body. Negative correlations with HR were found in the right medial and lateral OFC, right inferior frontal gyrus, right putamen and ventral insula, and bilateral temporal pole and parahippocampal cortex. Coordinates and statistics

[2]The same relationships were found after regressing out all MRI-related covariates within each subject ($p <= .0003$ for all tests), except that the first and second baseline periods were no longer significantly different (0.07 BPM, $t = 0.91$, $p > .10$).

for all Path *b* effects are reported in Table 2, and group-averaged time series data for key regions are shown in Figure 2.

At the second level, the slope of brain-heart connectivity (Path *b*) was positively moderated by HR reactivity in several regions, including the pgACC, rdACC, and left caudate (Table 2). That is, in these regions, the magnitude of an individual's HR reactivity to Speech Prep was correlated with the strength of the brain-HR coupling (controlling for SET). All of the regions listed above showed reliable first-level effects as well, indicating reliable brain-HR correlations in the group as a whole. In addition, another set of regions showed second-level moderation but did not show reliable brain-HR (Path b) correlation in the group. These regions may thus be associated with positive brain-HR connectivity in high HR responders, but negative brain-HR connectivity in low HR responders. These regions included the pre-SMA, superior frontal cortex, and additional areas of the right caudate.

Negative second-level correlations between brain-heart connectivity and HR reactivity included several areas showing negative brain-heart correlations at the first level: right OFC, right putamen, and left temporal pole (Table 2). Thus, these regions showed a negative time series correlation (Path *b*) that was stronger (more negative) in high HR responders. Additional negative moderation was found in other regions that did not show significant first-level effects, including left OFC, vmPFC, several temporal cortical regions, and brainstem regions including the midbrain and hypothalamus. Because the second-level moderation analysis tests the relationship between brain-heart regression *slopes* and HR reactivity, these regions showed negative brain-heart associations in the high HR responders, and positive brain-heart associations in the low HR responders.

ROI analysis of the amgydala

Notably, the pattern of results described above did not include the amygdala, which was recently implicated in a performance threat by Gianaros et al. (Gianaros et al., 2008a) and has been a focus of attention in studies of conditioned fear and other negative affective processes (Etkin & Wager, 2007; Phelps, Delgado, Nearing, & LeDoux, 2004; T. Wager et al., 2008; T. D. Wager et al., 2008). We performed ROI analyses on anatomically defined amygdala subregions (see Methods) including the left and right corticomedial group (superior amgydala) and basolateral complex. No positive associations with SET or HR (Path a or b) at either the first or second levels were found in any regions. All subregions showed significant or near-significant de-activations during Speech Preparation (negative Path a effects; see Table 3). Only in the right basolateral amygdala was brain activity predictive of HR, as indicated by negative Path b effects at first- and second-levels (Table 3). A trend towards significant mediation (a*b) was found in this area. Significant mediation was found in the right corticomedial group, but in the absence of significant average a and b effects, this finding indicates the presence of a reliable pathway that differs across individuals in whether increases or decreases lead to HR increases. The group-average time course plot from the right basolateral amygdala is shown in Figure 3. Overall, the results suggest that amygdala increases are not a feature of speech task-related SET, differentiating it from the performance threat results of Gianaros et al. (2008) and other paradigms that elicit negative emotional experience, such as conditioned fear.

Overlap of SET and HR effects (Path *a* and Path *b* conjunction)

The key regions that link social threat with HR changes should show both SET responses (Path *a*) and HR correlations controlling for SET (Path *b*; see Methods). Figure 3 shows the regions with significant results in both tests (at $q < .05$ FDR in each), along with their group-average time series (using methods described in (Lindquist, Waugh, & Wager, 2007); see the figure legend for details). Statistical details are listed in Table 4. In Figure 3, regions

showing positive first-level Path *a* and *b* effects in red, and negative first-level Path *a* and *b* effects in blue. The pgACC and right caudate head were the only regions that showed positive effects in both tests. The right OFC and putamen showed negative effects in both tests, indicating that they are de-activated by SET and that the degree of de-activation predicts HR increases across time.

Thus, the pattern of results presented thus far suggests that there are bi-valent effects of SET that are linked with HR increases, including activation in the pgACC and de-activation in the OFC and striatum. Notably, each of these regions showed second-level individual differences effects consistent with the first-level time series analyses. Strong HR "reactors" showed larger (more positive) SET-pgACC and pgACC-HR effects, and also larger (more negative) SET-OFC, OFC-HR, SET-putamen, and putamen-HR effects (see Table 1 and Table 2).

Does the brain track individual profiles of HR changes across time? Detailed analysis of pgACC, OFC, and putamen

We found reliable relationships between brain and HR in several key regions (pgACC, mOFC, and right putamen), both in time series correlations and in correlations with HR reactivity. However, there are a number of potentially confounding processes, such as internal sub-vocalization or cognitive planning activity, that are potentially correlated with HR and not captured completely by the box-car Speech Prep indicator variable. Thus, to more firmly establish the relationship between brain activity and HR, it is important to test how tightly coupled brain time series are with individual patterns of HR.

We first visualized the data in the key regions to assess the strength of the relationship between brain time series and HR changes across time. Detailed plots of the data for the pgACC are shown in Figure 4. Figures 4A and 4B show superimposed plots of the group-average brain and HR time series for the raw data and with covariates removed, respectively. Other regions showed similar data quality, but are not shown for space reasons. In addition, the relationship was apparent for individual participants, as shown in Figure 4C. Additional analyses, presented in Supplemental Analysis 1 (see Supplementary Material), demonstrated that brain responses tracked individual profiles of HR increases, and suggests that general task demands that vary across time are unlikely to explain the brain-HR relationships.

Multilevel mediation analyses of key regions

The full multi-level path model was tested on regions that showed both first-level SET (Path a) and HR (Path b) effects. This analysis provides only descriptive reports of statistics for the first-level *a* and *b* effects used to select voxels (since inferences on these regions was already provided by the voxel-wise search), but it provides new inferences on the significance of the formal mediation (*a***b*) effect and the second-level relationships with HR reactivity. The test of mediation amounts to a test of whether controlling for each brain mediator explains a significant amount of the co-variance between the SpeechPrep predictor and the HR time series (see Methods).

We tested both path models with only a single brain mediator (the pgACC, mOFC, or putamen) and a path model with all three regions entered as mediators in the same path model. The significance of the individual regions' path coefficients was qualitatively the same in both cases. For illustrative purposes, we show plots and statistics for the path model with the pgACC as the only mediator in Figure 5. The left panels (A) shows plots for the first- and second- level results for Path *a* ($P < .001$ at both levels), and the right panels (B) shows plots for Path *b* ($P < .001$ at both levels). The line plots in Figure 5 show first-level

regression slope estimates for each participant as blue lines, and the group average with its standard error in gray. The second-level moderation scatterplots show the relationship between path amplitude and HR reactivity. Notably, the mediation effect was significant ($a*b = 0.29$, $t(21) = 5.22$, $P < .001$), and second-level analyses showed that HR reactivity positively predicted Path *a*, Path *b*, and Path *a*b* slopes. That is, the functional pathway from SET to brain to HR was strongest in those with high HR reactivity.

The path model with all three regions entered as mediators is shown in Figure 6. This analysis was designed to distinguish between two alternative hypotheses. First, if the pgACC, OFC, and putamen are independent mediators of threat effects on the heart, then they should be significant mediators even when other regions are included (and their activity controlled for) in the model. Alternatively, activity in these regions could be part of a single bi-valent pattern or distributed "mode"; that is, each region may carry redundant information. If the dorsal and ventral MPFC are relatively specialized for sympathetic and parasympathetic control, respectively, as previous analyses have suggested (P. J. Gianaros et al., 2004;Lane, Reiman, Ahern, & Thayer, 2001), then we might expect the first case.

The results showed that each of the brain regions was an independent mediator of SET-HR covariance. As listed in Table 5, first-level *a* and *b* path coefficients remained significant for each of the three regions ($P < .05$ in all cases), and the first-level mediation effect was significant for each region as well ($P < .005$ in each). Thus, each region independently mediated some of the relationship between experimentally manipulated SET and HR profiles across time. At the second level, HR reactivity moderated the strength of all first-level effects ($P < .05$ in all cases), with the sole exception of a non-significant moderation of the putamen-HR partial path coefficient. Thus, the degree to which a participant was an overall autonomic "responder" moderated each of these functional SET-brain-HR pathways. Partial regression plots are shown in Figure 6 for the pgACC and mOFC.

In Figure 6, significant effects are shown as black arrows, and non-significant effects shown as light gray arrows. The intrinsic "effective connectivity" among these three regions was assessed by regressing each brain region on the others using the multi-level path modeling framework (see Methods), controlling for indirect effects of the SpeechPrep predictor and fMRI time series from other regions. These analyses showed that the pgACC was functionally coupled with both mOFC ($t = -6.53$, $p < .0001$) and putamen ($t = -6.26$, $p < .0001$), but mOFC and putamen were not significantly coupled ($t = 1.50$, $p = .15$). This suggests that there are functional relationships between the regions that show activation and deactivation in response to SET, but that these relationships are not sufficiently strong that the information about HR contained in the regions is redundant.

These path models do not take relative hemodynamic or neural latency differences across regions into account. We also conducted additional, supplementary cross-correlation analyses to examine the relative timing of signals in key regions. The results, shown in Supplementary Analysis 2 and Supplementary Figure S2 (see Supplementary Material), showed evidence that right mOFC activity preceded that of the other regions, consistent with an association between mOFC and fast, possibly predominantly parasympathetic effects on the heart. It is also worth mentioning that the optimal lag for brain-HR connectivity in each region was less than 300 ms (from zero-lag). Thus, correlation between brain and HR time series without convolution of the HR with an HRF signal is appropriate, and any convolution or lag is not likely to improve the strength of brain-HR connectivity estimates.

Discussion

Social status and perceived social and intellectual competence are extremely important factors in modern human life (Fiske, Cuddy, & Glick, 2007). Threats to self-esteem and negative evaluation—or social evaluative threat (SET)—relate to social status and are among the most potent laboratory and real-life stressors in contemporary society. Because SET often arises from a complex analysis of inter-personal relationships, rather than the presence of any particular simple sensory cue, it is likely to be generated by high-level appraisal processes involving the frontal cortex. Thus, SET is a good model for studying the cortical and subcortical brain networks associated with peripheral physiological changes. We used silent speech preparation, a component of a common laboratory stressor that produces robust HR responses (Berntson et al., 1994; Kirschbaum et al., 1993) and other clinically relevant cardiac events (Rozanski et al., 1988), as a SET manipulation during scanning.

In this study, we have found evidence for multiple independent mediators of social threat effects on HR. Specifically, we found that a particular pattern of cortical activity is tightly coupled with HR reactivity to speech preparation. This pattern includes reciprocal activation changes in two sub-regions of the MPFC—pgACC and vmPFC/mOFC—and the right putamen, a portion of the striatum. SET was associated with increases in pgACC (Path *a*), and those increases predicted HR variations across time (Path *b*). SET was associated with decreases in vmPFC/mOFC and striatum (negative Path *a*), and the larger the brain *decreases*, the higher the HR (negative Path *b*). These results suggest that changes in brain activity predict the evolution of HR responses to the SET challenge over time.

In addition, individual differences in SET-brain and brain-HR coupling predicted the magnitude of HR reactivity. This is evidenced by results from the second level in the multi-level path model. Stronger HR “reactors” expressed the pattern of increases in pgACC and decreases in vmPFC and striatum more strongly than non-responders (moderation of Path *a* by HR reactivity). Stronger HR “reactors” also showed stronger coupling between pgACC increases and HR and between vmPFC decreases and HR (moderation of Path *b* by HR reactivity). Overall, the pattern is consistent with the notion that some individuals did not show strong reactions, and thus showed little task-driven variability in both brain activity and HR. Combined, the within-subjects and between-subjects results provide evidence for a dual-process model of HR control in different medial prefrontal sub-regions.

The pgACC is a distinct subdivision of the anterior cingulate from the more ventral subgenual region and the more dorsal anterior mid-cingulate cortex according to Vogt (Palomero-Gallagher, Vogt, Schleicher, Mayberg, & Zilles, 2008; Vogt, 2005). The pgACC and rdACC are together associated with diverse emotional processes (T. Wager et al., 2008), such as in the context-driven modulation of emotion and pain (Petrovic et al., 2005; Porro, 2003; Wager, Scott, & Zubieta, 2007)(Faymonville, submitted). In a companion paper (Wager et al., submitted), we have replicated this basic finding in a separate sample, and have extended these results with several additional analyses, including analyses of brainstem mediators of the cortex-HR relationship. This study, conducted using a different fMRI pulse sequence, found comparable results in a slightly more dorsal area in the anterior portion of the anterior mid-cingulate cortex, which we refer to as the rostral dorsal cingulate (rdACC). Results in both studies were statistically quite strong and survived correction for multiple comparisons in multiple effects (Path *a*, Path *b*, and the mediation *a*b* effect). It is possible that the results from this study also showed effects in subgenual cingulate, as the activated region covers this area, but based on the weight of evidence from other studies, we believe that pgACC is the best overall descriptor.

Medial prefrontal subregions, emotion, and autonomic control

A wealth of non-human animal literature supports a connection between MPFC and autonomic control of the heart (Bandler et al., 2000; Barbas et al., 2003; Devinsky, Morrell, & Vogt, 1995; Saper, 2002), and MPFC has been referred to as "visceromotor cortex," in contrast to lateral orbital "viscerosensory" areas (Price, 1999). Interestingly, vmPFC has been linked to higher-order contextual control over stress responses as well, providing a conceptual link with the cognitive generation and regulation of stress responses in humans. For example, a recent series of studies has shown that rat vmPFC inactivation abolishes the beneficial effects of stressor controllability on fear responses (Amat et al., 2005), whereas vmPFC activation mimics the effects of control (Amat, Paul, Watkins, & Maier, 2008). vmPFC inactivation also blocked the "immunizing" effects of prior exposure (Amat, Paul, Zarza, Watkins, & Maier, 2006) on stressor reactivity. Another series of experiments has shown that inactivation of the vmPFC or hippocampus prevents consolidation of fear extinction (Corcoran & Quirk, 2007b; Sierra-Mercado, Corcoran, Lebron-Milad, & Quirk, 2006), which is considered to be another type of safety-related context learning (Davis, 1992). Conversely, vmPFC stimulation potentiates or mimics extinction memory (Milad & Quirk, 2002; Milad, Vidal-Gonzalez, & Quirk, 2004).

Recent neuroanatomical, lesion, and electrophysiological evidence also supports a functional distinction between dorsal and ventral MPFC sub-regions (Quirk & Beer, 2006). A potential rat homologue of the pgACC/mOFC distinction is the difference between the dorsal pre-limbic (PL) and ventral infralimbic (IL) medial frontal cortices. These two structures project to different subcortical nuclei (Gabbott, Warner, Jays, Salway, & Busby, 2005; Hoover & Vertes, 2007; McDonald, Mascagni, & Guo, 1996; Vertes, 2004)—the PL to the basolateral amygdala, associated with fear learning, and the IL to intercalated inhibitory neurons in the amygdala (Vertes, 2004), which are associated with the suppression of fear behavior during extinction. It is IL in particular in which stimulation potentiates fear extinction and lesions reduce it (Milad & Quirk, 2002; Quirk, Russo, Barron, & Lebron, 2000). In addition, stress induces dendritic retraction in IL (but not PL), an effect associated with impaired fear extinction (Izquierdo, Wellman, & Holmes, 2006). IL lesions also reduce stress-induced activity in hypothalamic pre-autonomic neurons, whereas PL lesions do not (Radley, Arias, & Sawchenko, 2006). Instead, PL activity is related to the expression of learned fear responses (Corcoran & Quirk, 2007a). All of the abovementioned information is consistent with the view that PL activity promotes autonomic responses to stress, perhaps by mediating cognition and memory-related processes (Gabbott, Warner, Jays, & Bacon, 2003; Vertes, 2006), whereas IL activity inhibits them. The general pattern across these results matches the pattern of reciprocal control of HR in our study.

The pattern of reciprocal dorsal and ventral predictors of HR (though "dorsal" in this case is still relatively ventral, in the pgACC) replicates and extends findings from earlier human neuroimaging papers of stressful tasks. For example, in a large study with nearly 100 participants, Gianaros et al. (P. Gianaros et al., 2004) found positive and negative correlations with HR3 in the dorsal MPFC and vmPFC/mOFC, respectively. Critchley et. al (Critchley et al., 2000), in an important early study, investigated correlations between brain activity and HR and blood pressure increases with mental arithmetic stress and hand exercise. Increases during the two "stressor" tasks were found in the dorsal cingulate, and increases were positively correlated with blood pressure. Conversely, OFC showed decreases with the stressor tasks, and mOFC in particular was negatively correlated with blood pressure.

[3]Heart period, 1/HR, was actually analyzed, which has a more nearly linear relationship with underlying sympathetic and parasympathetic effectors (Cacioppo et al., 1994), but we discuss the results in terms of heart rate here for ease of interpretation.

A similar dorsal/ventral distinction has emerged in studies of fear conditioning in humans and other animals, supporting the idea that these different MPFC sub-regions play opposing roles in the generation of affective states. Several human fear conditioning studies have found increases in the dorsal MPFC and reductions in mOFC when cues associated with shock (conditioned stimuli) are presented (Delgado, Nearing, Ledoux, & Phelps, 2008; Phelps et al., 2004; Schiller, Levy, Niv, LeDoux, & Phelps, 2008). In rats, PL shows increases electrophysiological responses during trace conditioning to aversive cues, whereas activity in the IL cortex decreases during fear conditioning (Gilmartin & Mcechron, 2005).

Though we did not perform tests of lateralization, our findings of right-sided effects in mOFC are consistent with work showing right-lateralized increases in HR reactivity with sodium amytal inactivation (Ahern et al., 2001) and vmPFC damage (Hilz et al., 2006). The current findings extend this literature by showing this reciprocal relationship between dorsal and ventral subregions of the mPFC in response to social threat, as well as showing that although there is a functional coupling between these two regions, their effects on HR reactivity are independent of each other. Our findings are also consistent with the idea that dorsal and ventral MPFC subregions may play preferential roles in sympathetic and parasympathetic regulation of the heart (and possibly other organs). This idea is discussed in more detail in the Supplementary Discussion.

HR reactivity as an individual differences measure

Our results also have implications for understanding individual differences in threat and stress reactivity. In our study, the degree to which an individual expressed each of the links between SET, brain activity, and HR strongly predicted the degree of HR reactivity to the challenge. HR reactivity provides a robust way of characterizing individual differences in physiological responses to stressors, rather than relying solely on subjective reports as outcome measures, which can depend on self-presentation (Weinberger, Schwartz, & Davidson, 1979) and generally have more complex determinants. HR reactivity as an individual difference measure has been associated with increased hypothalamic-pituitary-adrenal axis activity (al'Absi et al., 2000; Cohen et al., 2000; Sgoutas-Emch et al., 1994; Uchino et al., 1995) and cellular immune reponses to acute psychological stressors (reviewed in (Cacioppo, 1994))(Knapp et al., 1992; Sgoutas-Emch et al., 1994; Uchino et al., 1995). HR reactivity to acute laboratory stressors in particular has been associated with blastogenic responses to mitogen (a probe of immune cell proliferation in response to challenge), NK cell counts, and lymphocyte cell counts (t-cells, b-cells) (Brosschot et al., 1992; Cohen et al., 2000; Knapp et al., 1992; Landmann et al., 1984; Sgoutas-Emch et al., 1994; Uchino et al., 1995). HR reactivity is an outcome deserving of study in its own right, and is not likely to be highly related to reported subjective anxiety or other psychological reports. In the present study, for example, HR reactivity was uncorrelated with emotional resilience, optimism, and other measures.

Absence of amygdala increases in SET: Differentiation of SET from other negative emotional tasks—Though reciprocal dorsal and ventral MPFC activity has emerged as a common theme across manipulations of emotion, the present results also provide evidence that not all aversive emotional states and outcomes are the same. For example, the basolateral and central amygdala have been strongly implicated in the learning and expression of cue-threat associations, respectively (LeDoux, 1996). Amygdala activity is a prominent correlate of anticipatory anxiety (Nitschke et al., 2009), conditioned fear in anticipation of shock (Phelps et al., 2004), and reported negative emotion in response to affective pictures (Ochsner & Gross, 2008; Ochsner et al., 2004; Phan et al., 2005). Amygdala hyperactivity is a prominent feature of multiple anxiety disorders (Etkin &

Wager, 2007), and its neurons encode both positive and negative predicted value (Paton, Belova, Morrison, & Salzman, 2006).

However, our speech preparation task evoked no detectable increases in the amygdala—rather, they showed evidence for decreases during SET that did not strongly predict HR. Flexible change-point models that could have detected activation that was uncoupled from the task demand and HR also showed no evidence for amygdala responses during the speech preparation period (Lindquist et al., 2007). The lack of amygdala activation is consistent with other studies of performance stressors, some of which have produced reliable *decreases* in the amgydala and hippocampus in both PET and fMRI (Dedovic et al., 2005; Pruessner et al., 2008). Physical pain shows a similar pattern of amygdala unresponsiveness or decreases (Derbyshire et al., 1997; Petrovic, Carlsson, Petersson, Hansson, & Ingvar, 2004), though results vary across studies. This pattern contrasts with recent reports of increased amygdala responses in cardiovascular responders performing a stressful cognitive task (Gianaros et al., 2008b), perhaps due to differences in the nature of the stressor; future research should address this issue. The latter study notwithstanding, the brain data in this study support the notion that SET responses are qualitatively distinct from some other forms of affective processing.

Challenges with physiology-induced imaging artifacts

One potential problem with any study relating brain activity to HR, blood pressure, or emotional states that induce physiological changes is the possibility that the psychological demands might induce vascular changes and thus fluctuation in fMRI signal that are non-neuronal in origin. There are several lines of defense against interpreting the results of the present study as vascular artifacts.

First, the pattern of results across the brain is very specifically localized to regions known to be involved in autonomic control from converging lesion, electrophysiologal and neuroanatomical studies in animals and humans. Second, that argument notwithstanding, one way to assess whether this pattern is artifactual is to examine whether studies of mechanical changes in vascular responses produce similar results. The evidence to date suggests that they do not. For instance, hypercapnia (increased blood CO2 induced, for example, by breath-holding) results in increases in fMRI signal throughout the brain, rather than in specific regions (Thomason, Burrows, Gabrieli, & Glover, 2005; Vazquez et al., 2006). The pgACC and mOFC show relatively low changes in percent signal during a breath-hold challenge relative to other regions of the brain (see Thomasen et al., Figure 6). Third, many kinds of physiological artifacts, such as effects of pulsatile motion due to the heart-beat, occur at different temporal frequencies from neuronal-induced fMRI signal, and are not likely to be significant sources of the brain-HR covariance observed here (see Birn et al. this issue). Finally, in our second, companion study (Wager et al., this issue) we were able to identify and control for signal in major arteries, including the anterior cerebral artery at the genu of the corpus callosum, and the MPFC findings were replicated.

Other standard caveats concerning the use of path models to infer directionality (i.e., causality) of the results apply. The use of a directional path model that specifies brain activity as a predictor of HR does not provide strong evidence that brain activity causes HR changes, though in this case the location of findings in “visceromotor” regions of MPFC (Price, 1999), and the findings that the neuronal activity underlying fMRI responses was likely to occur 5–6 sec before observable HR changes, favor a brain-to-heart causal directionality.

Conclusion

In conclusion, these findings contribute significantly to the investigation of brain-physiology relationships in the context of social threat. The relationship between social threat and associated physiological responses (HR) was mediated by reliable and sustained increases in pre-genual cingulate/MPFC and decreases in vmPFC/mOFC. Future investigations should more specifically examine whether the dorsal/ventral distinction within the MPFC maps onto distinctions within the peripheral nervous system (sympathetic and parasympathetic, respectively), their relationships to emotion reports (negative and positive, respectively), and their potential subcortical mediators. We address some of these issues in a companion paper (Wager et al., submitted).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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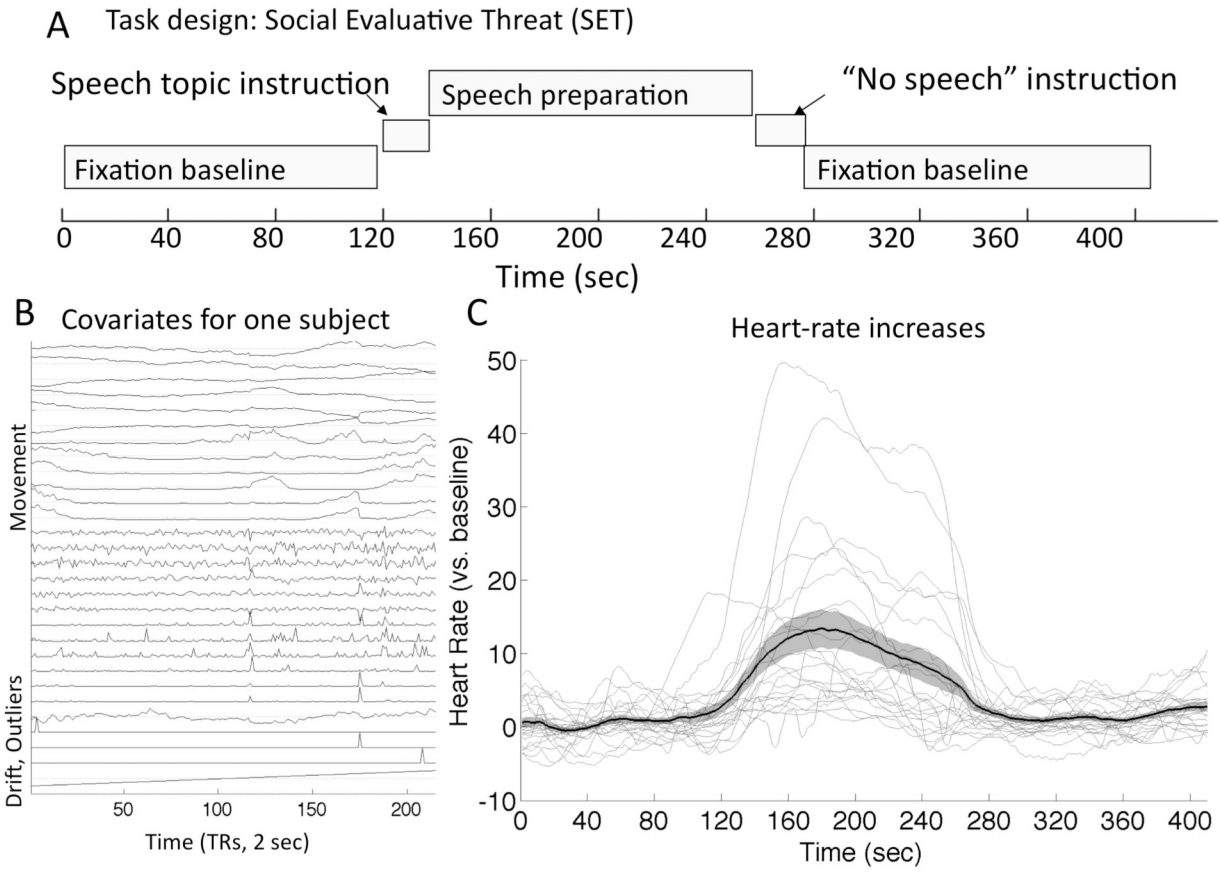

Figure 1.

A) Task design. The SET manipulation involved a 2-min resting baseline, a 15 sec visual presentation of the speech topic, a 2-min preparation period, a 15-sec no speech instruction, and a 2.5 min recovery period. B) Nuisance regressors for fMRI analysis. These varied across participants, but always included regressors for linear and higher-order head movement, potential outliers based on task- and physiology-blind global signal analysis, global activity values, and linear drift. C) Heart rate changes over time. Individuals are shown by the light gray lines, and the group average (with shaded standard error region) is shown by the heavy black line.

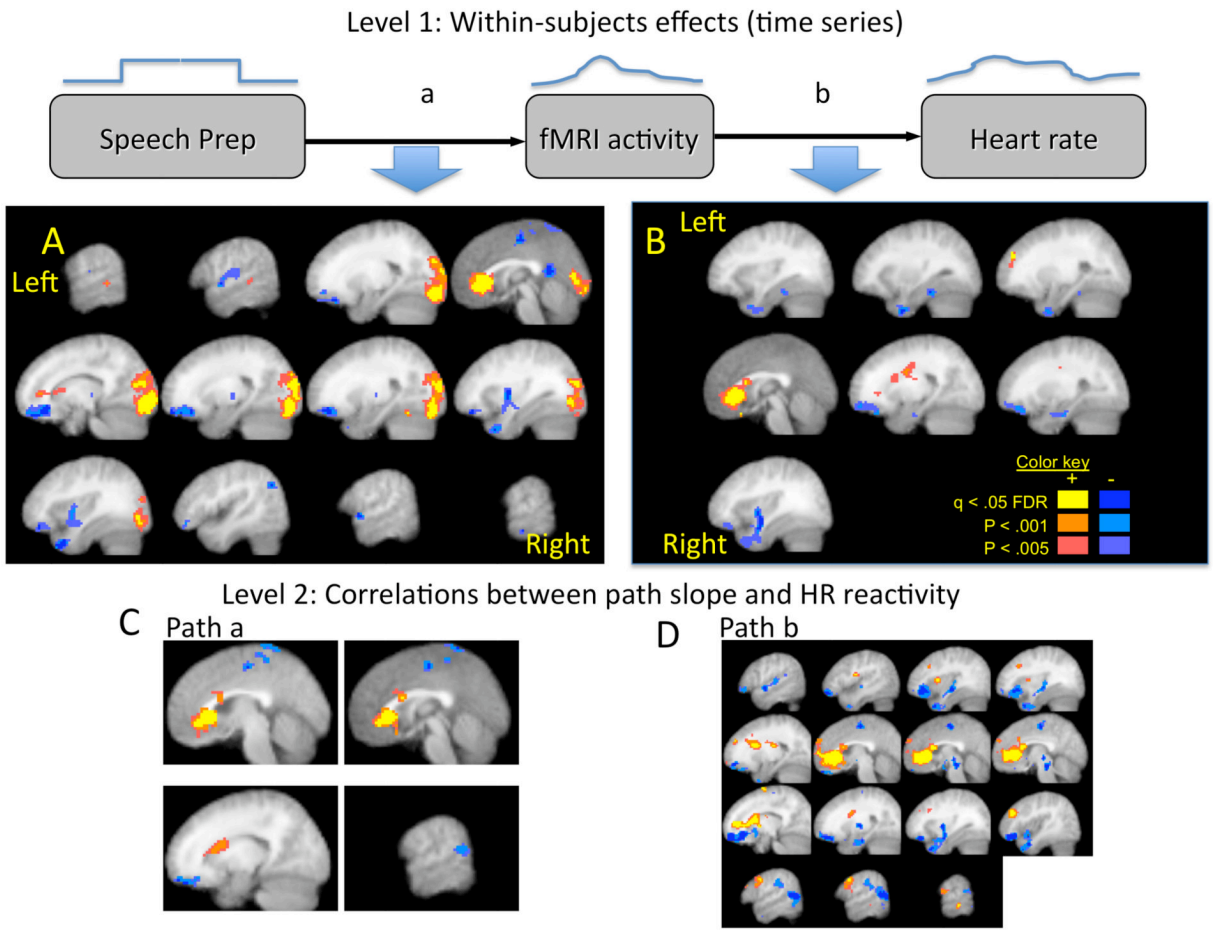

Figure 2.

Path model (top) and results of the multi-level mediation effect parametric mapping search. Relationships between Speech Prep and brain activity (Path a) and between fMRI activity and heart rate (Path b, controlling for the Speech Prep regressor) were tested both within person and between persons, with heart rate (HR) reactivity as a predictor of individual differences in path amplitude. A) Path a results for the first-level model (time series). Saggital slice showing regions whose activity increased (yellow/orange) or decreased (blue) in response to the social evaluative threat (SET) challenge. Significant regions of 3 or more contiguous voxels at $q < .05$ False Discovery Rate corrected, and contiguous regions at $p < .005$, are shown. B) Path b results for the first-level model (time series). Sagittal slices showing significant positive (yellow/orange) or negative (blue) correlates of heart rate changes over time, controlling for Speech Prep regressor. C) Path a results for the second-level model, showing correlations between SET-brain connectivity and HR reactivity. Positive correlations are shown in yellow, and negative correlation in blue. D) Path b results for the second-level model, showing correlations between brain-HRconnectivity and HR reactivity.

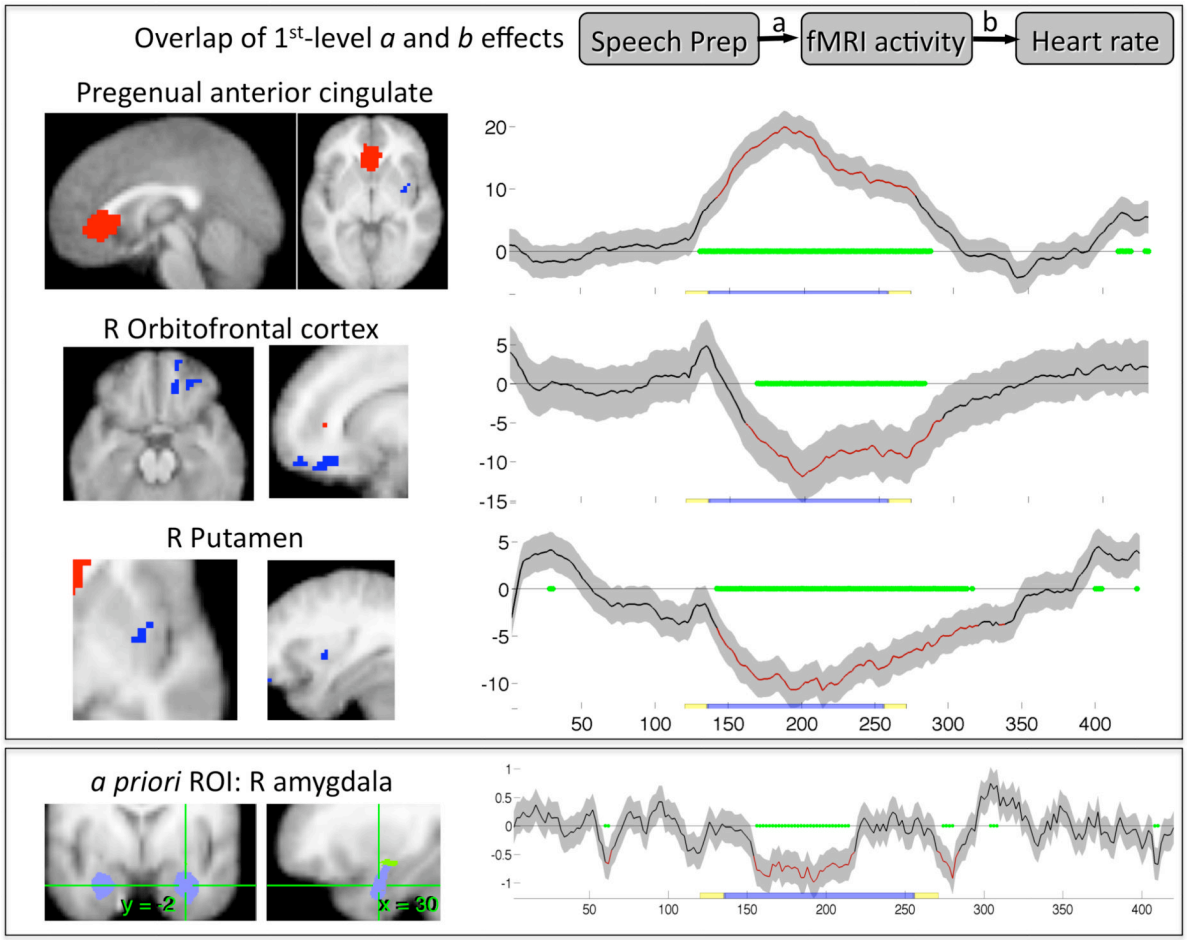

Figure 3.

Regions showing both Path a (SET responses) and Path b (prediction of HR) in the first-level (time series) model, and results from one amygdala region of interest (ROI). Positive results for both Path a and Path b are shown in red, and negative results for both are shown in blue. No regions showed positive a and negative b effects or vice versa. Results are shown at P < .001 for display, but all regions showed significant effects in both paths at q < . 05 FDR (p < .0004). The time series plots at right show group-averaged time series data across the run estimated with the Hierarchical Exponentially Weighted Moving Average (HEWMA) model. They are similar to, but smoother, than the raw data averages. Instruction periods are shown as yellow horizontal bars, and the Speech Prep period is shown as a blue horizontal bar in each plot. The HEWMA model provides estimates of which time points are deviant from the pre-SET baseline using a 2-state mixture model; these periods are marked with a red line. Time points that were individually significantly different from the average pre-scan baseline (zero on the y-axis) are marked with green dots at y = 0. The right (R) putamen alone showed evidence for a trend towards de-activation even before the speech instruction onset, as evidenced by a change-point value that occurred before the instruction. The right amygdala ROI showed significant de-activation in response to SET, but this activity did not predict heart rate fluctuations. Other amygdala sub-regions showed similar results.

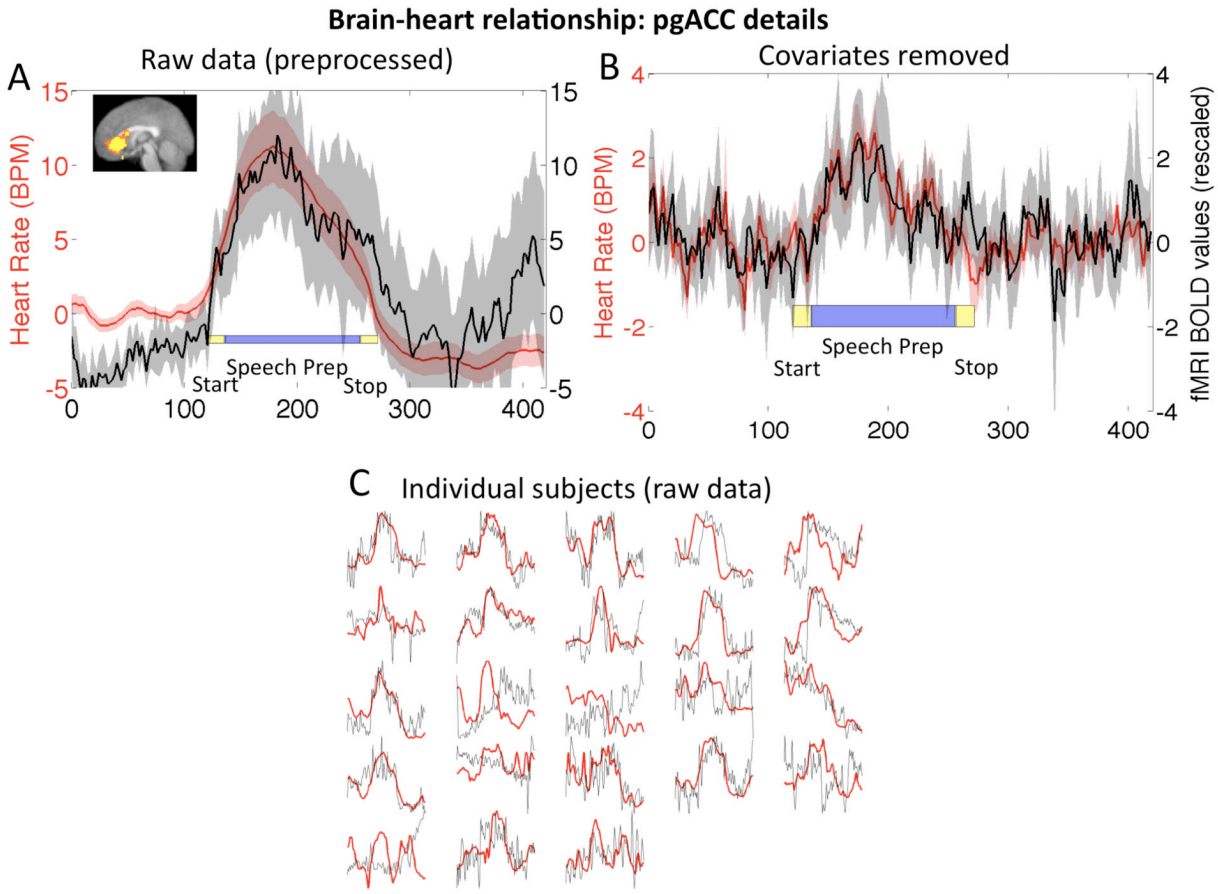

Figure 4.

Visualization of brain-HR connectivity (related to Path b) for the pregenual cingulate. A) Superimposed plots of the group-average time series data (black, with standard error regions shaded) against the group-average HR data (red). Speech Prep-related variance was not removed for display purposes, so the response to the social threat challenge can be seen. In addition, nuisance covariates (see Figure 1C) were not removed for display purposes, so the original response in both brain and heart can be seen. Only standard preprocessing procedures were performed. Instruction periods are shown as yellow horizontal bars, and the Speech Prep period is shown as a blue horizontal bar. B) The same plot as in (A), but with nuisance covariates removed. C) Plots of brain (black) and superimposed HR (red) for individual subjects, each shown in a separate panel.

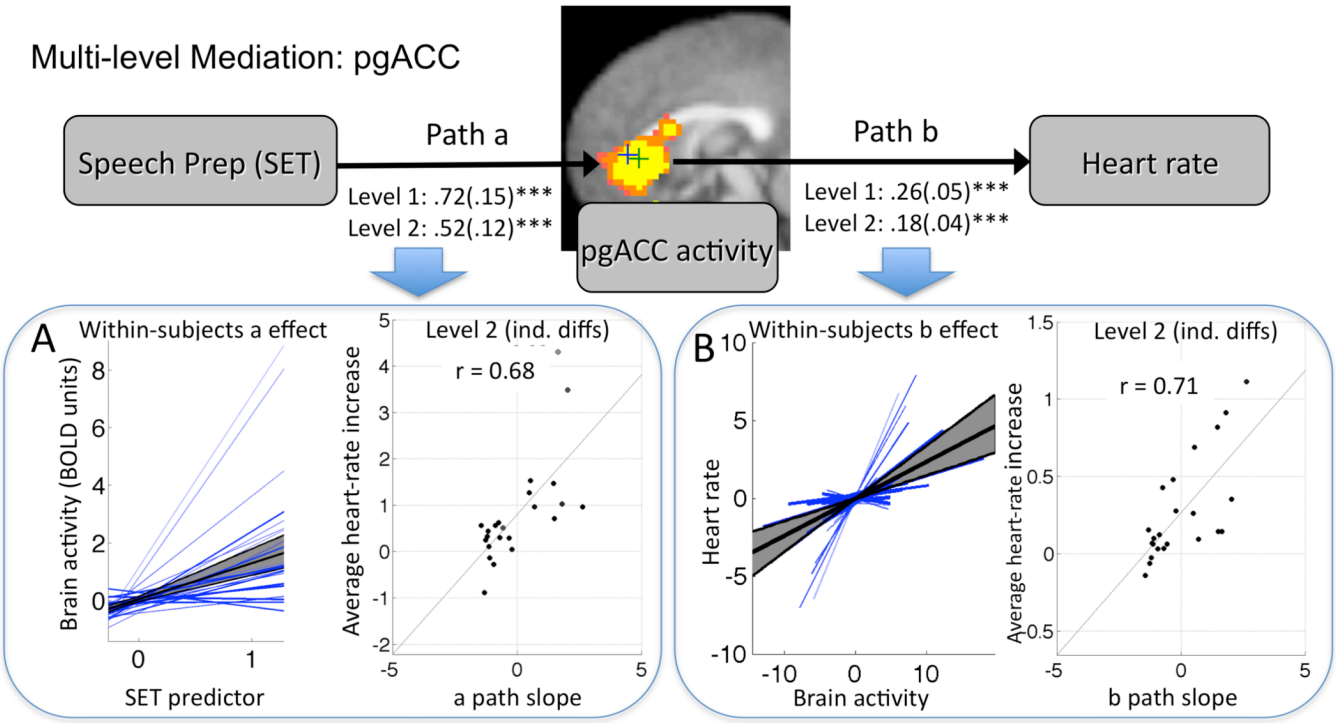

Figure 5.

Path diagram and effect plots for the pregenual anterior cingulate (pgACC). A) Path a results for Level 1 (time series SET-brain relationship) and Level 2 (correlation between SET-brain relationship and HR reactivity). Cross-hairs indicate center-of-mass coordinates for the SET effect (Path *a*, blue) and heart-rate prediction effect (Path *b*, green). Mean path coefficients are shown, with standard errors in parentheses. ***, P < .001. The line plot (left panel) shows the first-level effects, the relationships between the SET predictor (which took on values of 0 for baseline and 1 during speech preparation; x-axis) and fMRI activity (y-axis). Relationships for Individual participants are shown as blue lines, one per participant. The group-average effect with its standard error is shown by the black line and gray shaded area. The right panel shows a scatterplot of the second-level relationship between individual differences in the slope of the Path *a* effect (x-axis) and the average HR response to the task (y-axis). The significant relationship (r = .68, p < .00037 [the FDR threshold]) indicates that those with high HR reactivity showed larger SET-brain (Path *a*) effects. B) The same relationships for the brain-HR relationship (Path *b*), controlling for the SET predictor. Significant first- and second-level effects demonstrate the reliable link between individual profiles of brain activity and individual profiles of HR changes across time.

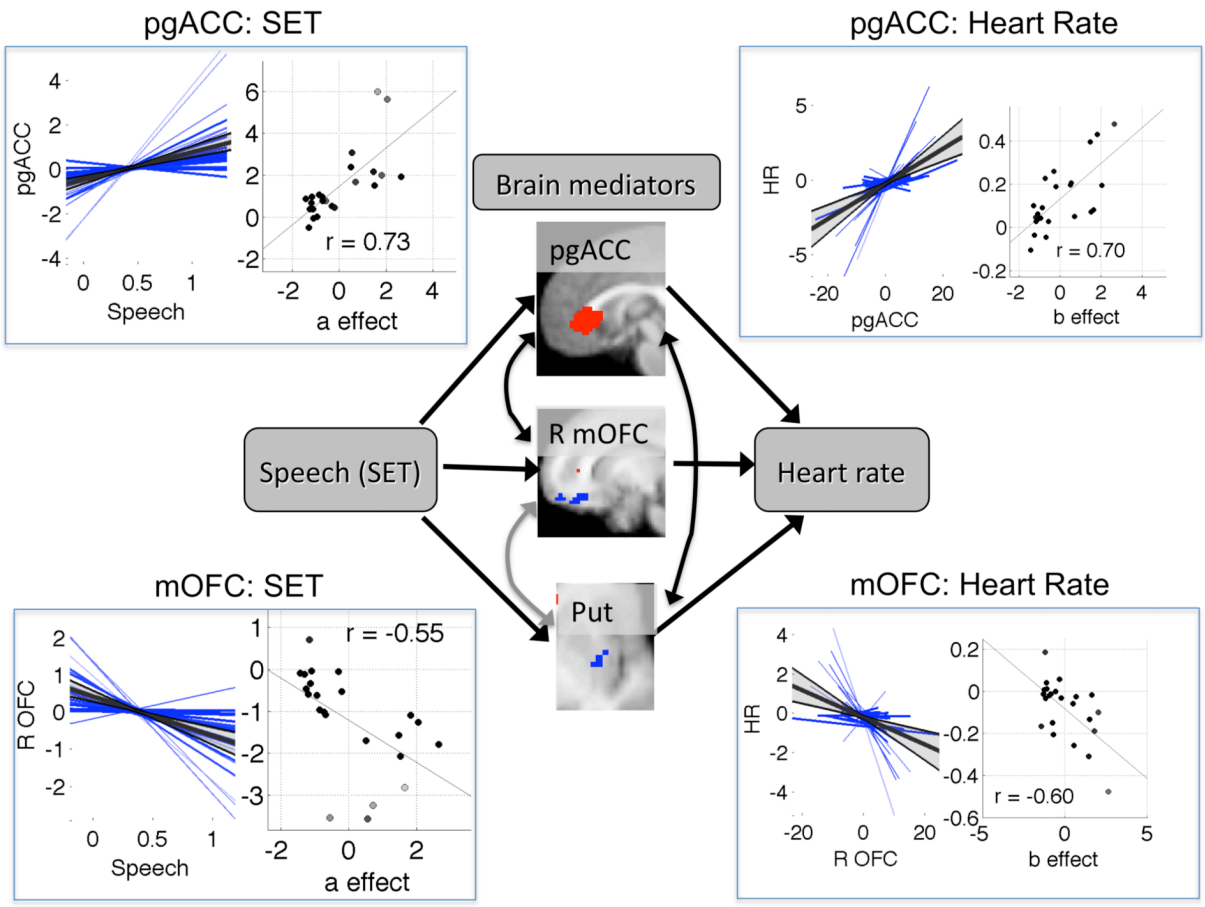

Figure 6.
Mediation path diagram for all three key mediators of social evaluative threat (SET) effects on heart rate. Solid black lines indicate significant relationships, and light gray lines indicate non-significant relationships. Connections hypothesized to be directional are shown as one-way arrows, whereas effects likely to be bi-directional (feedback loops) based on anatomy are shown as double-headed arrows. Causality could only be inferred for the SET-brain effects because SET was experimentally manipulated. First-level effects (SET-brain connectivity for Path a or brain-HR connectivity for Path b) are shown as line plots, and second-level effects (correlations between Path a or Path b and HR reactivity) are shown as scatterplots. Effect plots are shown for pregenual cingulate (pgACC) and medial orbitofrontal cortex (mOFC), but are omitted for putamen (Put) for space reasons. Full statistics are presented in Table 5.

Table 1

Brain responses to SET challenge (Path a)

		1st-level: within-subjects					*>2nd-level: moderation by average HR*				
Group	**Name**	**x**	**y**	**z**	**Vol. (mm3)**	**z**	**x**	**y**	**z**	**Vol. (mm3)**	**z**
Increases											
Medial frontal	pgACC	0	31	3	7383	5.15	−3	28	0	6387	4.46
Temporal	vTC	25	−56	−18	29	3.64	28	−56	−18	234	3.55
	STS	−66	−41	0	29	3.63					
Occipital	OCC	3	−88	−3	25986	5.29					
	L OCC	−25	−94	18	1963	3.71					
	R OCC	22	−91	30	59	3.54					
Decreases											
Orbitofrontal	L mOFC	−19	38	−21	117	3.48	−19	56	−21	176	3.43
	R mOFC	19	38	−15	1553	4.25	19	47	−18	1699	3.43
	R aOFC	22	59	−18	146	3.73					
Basal ganglia	R Putamen	34	0	6	293	3.88	31	−3	3	234	3.41
Insula	R valNS	44	9	−9	88	3.56					
Temporal	R TP	38	16	−39	410	4.05					
	R ITG	66	−19	−21	59	3.50					
	R STG	59	6	−3	117	3.88					
	L STG	−56	−3	0	29	3.40					
Diencephalon	Thal	9	−25	9	29	3.46					
	R SPL	44	−66	36	117	3.86					
Posterior medial	PCC	0	−53	15	850	3.96					
	MidCing	3	−16	57	703	4.13					
	Precun	−9	−59	69	29	3.44					
	Precun	3	−50	72	88	3.45					
Sensorimotor	SMC	6	−28	72	88	3.74					
	SMC	−6	−31	78	59	3.40					

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Table 2

Correlates of HR, controlling for SET (Path b)

		1st-level: within-subjects					2nd-level: moderation by average HR				
Group	**Name**	**x**	**y**	**z**	**Vol. (mm3)**	**z**	**x**	**y**	**z**	**Vol. (mm3)**	**z**
Positive association							**B level 2**				
Medial frontal	pgACC	0	28	0	7793	4.17	0	31	6	42305	4.23
	vmPFC	0	22	−24	29	3.38					
	vmPFC	−3	38	−21	29	3.47					
	rdACC	3	16	18	146	3.48	9	22	33	352	3.40
	rmPFC	−12	59	3	29	3.38					
	preSMA						16	−3	72	615	3.68
Lateral frontal	L DLPFC	−25	50	30	205	3.79					
	L SFG						−25	50	27	820	3.57
	R DLPFC						44	34	30	3340	3.68
Basal ganglia	R Cau	12	6	6	234	3.96					
	R Cau	19	−21	21	29	3.40					
	L Cau	−16	16	21	59	3.38	−22	−19	21	1172	3.42
	R Cau	19	−6	24	29	3.56					
Negative association											
Orbitofrontal	R mOFC	19	34	−24	29	3.51					
	R mOFC	16	41	−21	205	3.78	25	44	−15	9814	3.97
	R lOFC	31	41	−18	59	3.43					
	R aOFC	16	56	−15	176	3.50					
	L lOFC						−38	34	−12	6592	3.72
	L aOFc						−22	53	−15	762	3.97
Medial frontal	VMPFC/mOFC						−3	50	−27	498	3.38
Posterior medial	MidCing						3	−16	57	1934	3.97
Lateral frontal	R IFG	44	31	−12	29	3.40					
	R IFG	44	38	−3	176	3.78					
Temporal	L TP	−28	3	−45	322	3.73	−34	6	−39	3721	3.95
	L TP	−41	3	−42	29	3.40					

		1st-level: within-subjects					2nd-level: moderation by average HR				
Group	**Name**	**x**	**y**	**z**	**Vol. (mm3)**	**z**	**x**	**y**	**z**	**Vol. (mm3)**	**z**
	L STG						−50	−22	6	6240	3.74
	R TC/OCC						56	−56	9	4893	3.98
	L PHCMP	−31	−34	−18	29	3.39					
Basal ganglia	R Putamen/vINS	38	−3	−9	820	3.95	34	3	−21	8789	4.03
Insula	R vaINS	44	6	−15	117	3.51					

Table 3

Overlap of 1st-level SET and HR (a and b paths)

		x	y	z	Vol. (mm3)
Positive a and b paths					
Medial frontal	pgACC	0	31	3	5420
	R Cau	12	16	9	29
Negative a and b paths					
Orbitofrontal	R mOFC	16	41	−18	117
	R aOFC	19	59	−18	59
Basal ganglia	R Putamen	34	0	0	59